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HEAVY METALS LEVELS IN RAW COW'S MILK AND HEALTH RISK IMPLICATION FROM THERMAL POWER PLANTS EMISSION IN OBILIC, KOSOVA

SUMMARY

Contamination of food reflects the most serious consequences of environmental contamination, whereas cow's milk is considered the most representative food source contaminated with heavy metals. The main objective of this study is to evaluate the level of toxic heavy metals (Cd, Pb) and nutrition elements (K, Na, Ca, Mg, Cu, Fe, Cr, Zn) in cow milk collected from different sites in the Industrial area of thermal power plant in Obilic, Kosova. To determine the level of presence of chemical elements, total of 153 cow milk samples are collected from 51 cows. From each location sites during the morning milking in the different seasons [spring (phase I), summer (phase II), and autumn (phase III)]. Elements were determined using SAA atomic spectrometry. The results of this study indicate that heavy metals content extend the recommended values by International Dairy Federation and Codex Alimentarius Commission in all three phases of monitoring, and also there is a strong correlation between heavy metals and nutritional elements in cow's milk.

Keyword: heavy metals, nutrition elements, milk, correlation

INTRODUCTION

Due to its medicinal and dietary properties, the consumption of cow's milk is very popular in the world. This is also because the beneficial health effects are beyond its value. However, very little is currently known about the distribution, behavior and effects of trace elements in cow's milk. Industrial progress and the increase in agricultural production cause the use of large amounts of chemicals even in the production of animal feed, therefore agricultural production has

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become a permanent source of external chemicals for living organisms (Zeneli *et al.,* 2013). An important "direct indicator" of the uncontaminated condition of milk is determination of residual metal concentrations in milk. More ever, it can be also an "indirect indicator" of the degree of pollution of the environment from origin of milk (Licata *et al.,* 2004, González-Montaña *et al.*, 2012). The problem with metals is their ability to bioaccumulate, and in recent decades, contamination of milk with metals has been considered a dangerous problem. Heavy metals come into contact with animals through air, water and food, where after ingestion of unhealthy foods is considered the main source of metal residues in secreted milk.

Therefore, milk as a secretion of the mammary gland together with the many substances it carries, constitute a risk factor for the health of the consumer. Milk and most of the dairy products from cows are likely to be exposed to heavy metal contamination during lactation period (Ogut *et al*., 2016). In particular, metal residues in milk remain a concern due to consumption by infants and children.

Lead and cadmium cause the greatest concern in terms of adverse effects on human health (Zeneli *et al.,* 2008). The risk is also increased by the fact that they are easily transferred through food chains and are not known for essential biological functions. Therefore, the concentration of these two metals should be observe in cow's milk to guarantee the health of people (Fathi *et al*., 2020). On the other hand, milk is known as an excellent source of calcium and can supply zinc, iron and copper in smaller amounts (Pennigton *et al*., 1995).

Although the excretion of metals through milk is very low, the accumulation of their ecosystem (water-soil-plant-animal) makes them very toxic and harmful to us living organisms (Tunegova *et al.,* 2016).

According to conducted research's, milk and milk products contain around 38 micro and different trace elements. Some of them are essential and very important, because they are cofactors in many enzymes and play an important role in many physiological functions of humans and animals. The amount of metals in uncontaminated milk is undoubtedly small, but their content can be significantly altered and cause serious health problems (Amer *et al*., 2021). Depending on several factors, the content of these minerals in raw cow's milk can vary, e.g. lactation period, presence of any disease, seasonal variations, climatic conditions, annual feed composition and environmental pollution (Licata *et al.*, 2004, Yahaya *et al*., 2010). Therefore, data and research related to the concentration of heavy metals in cow's milk is important in assessing the risk to human health. Cadmium and lead accumulation in ruminants induce toxic effects in cattle, therefore also in humans who consume contaminated food (including milk) with toxic metals (González-Weller et al., 2006, Cai *et al*. 2009, Vromman *et al*., 2008).

According to previous studies, it was determined that the content of selected pollutants in the study area differ among elements and samples, and they can cause harmful effects in plants, animals and humans (Bajraktari *et al*. 2019, Zeneli *et al.,* 2011). Therefore, the aim of this study was to determine the residue levels of toxic heavy metals (Cd, Pb) and nutrition elements (K, Na, Ca, Mg, Cu, Fe, Cr, Zn) in Industrial area of Obilic, Kosova. It should be noted that toxic metal contamination in milk has been proven also in different countries.

MATERIAL AND METHODS

A total of 153 fresh cow milk samples (each sample 500 mL) were collected from 51 cows in villages selected in area of Kosovo Thermal Power Plants in Obilic Municipality (Figure 2). Samples was taken during the morning milking directly in sterile bottle to prevent any contamination. The milk samples from cow were carried in three phases (spring, summer and autumn). Transportation of samples into laboratory was carried immediately (at 4 °C).

Before the procedure, the working glassware are wet with detergent, rinsed with tap water. The same are moistened with 15% nitric acid, rinsed with distilled water and stored in the oven at 110 ° C as needed. From each sample, 0.5 g of each milk samples was taken and digested with 5 mL of concentrated nitric acid. Content of flask was heated at 80 ° C, until the clear digest was gained. The excess acid was evaporated. Cooling take place at room temperature, and the final solution was dilute to 25 mL with 0.2 mol/L nitric acid and filtered. Quantitative determination of selected elements and were conducted by using atomic absorption spectrometer (Jen *et al*., 1994). Also, blank solutions were also analyzed with same method. Data were statistically evaluated and concentrations were expressed as mean ± standard deviation, minimum and maximum values.

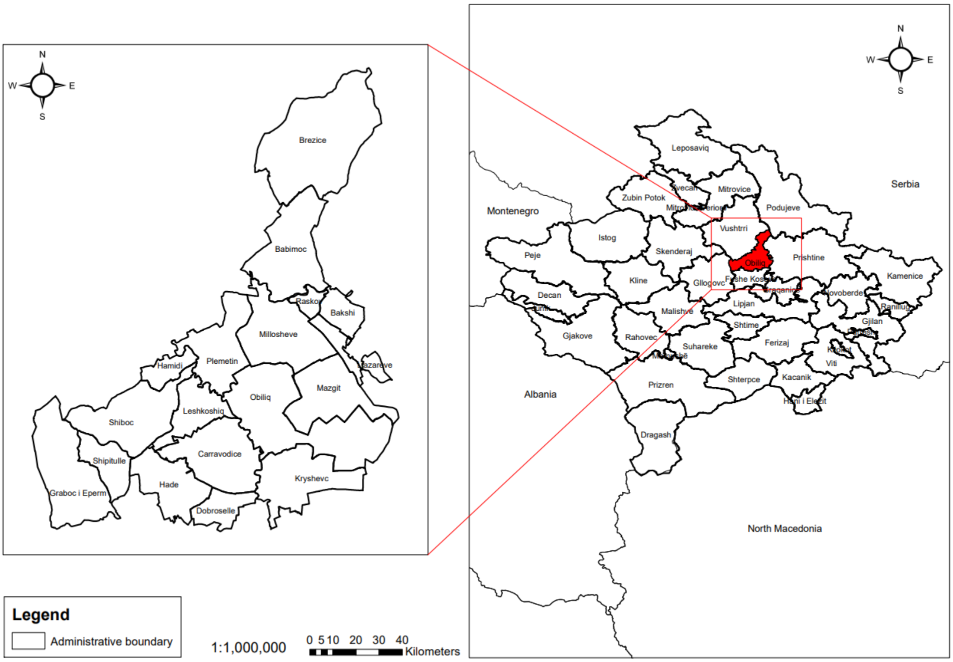

Figure 1. The map of Kosova and the Municipality of Obilic - The location of the Kosovo Thermopower Plants

RESULTS

The concentration of nutritional elements in milk samples during three phases showing the minimum, maximum, and average values for the analyzed elements in each phase are given in in Table 1, 2 and 3. Regarding the mean values of nutrition elements, they differ from sample to sample, and from one phase to another one. Iron mean concentration in phase one was 0.587 mg/L with minimum concentration 0.314 mg/L and maximum concentration 0.836 mg/L, which are lower compared with Phase II detected iron in milk samples. In phase two the mean iron concentration was 0.716 mg/L, with minimum concentration 0.218 mg/L and maximum concentration was 2.5 higher compared with phase I (2.042 mg/L). In phase three mean iron concentration was 0.467 mg/L, with minimum concentration lower than in phase one and two (0.047mg/L) and maximum concentration 1.041 mg/L. Copper mean concentration as nutritional elements varies from phase to phase, with lowest mean concentration [0.297 mg/L (phase I); 0.361 mg/L (phase II); and 0.170 mg/L (phase III)]. Maximum concentration was detected in phase II (5.085 mg/L) and lowest in phase III (0.59 mg/L).

Table 1. Mean, minimum, and maximum contents of selected elements in milk samples – Phase I

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Mean ± SD	0.587±0.15	0.297±0.08	0.307±0.12	3.064±0.41	0.291±0.11	33.909±5.11	9.758±1.28	20.217±1.82	0.318±0.11	1.293±0.52
Median	0.622	0.331	0.352	3.05	0.292	34.486	9.818	19.808	0.34	1.429
Minimum	0.314	0.156	0.019	2.337	0.159	22.701	6.705	17.25	0.104	0.144
Maximum	0.836	0.429	0.648	4.155	0.914	42.138	12.174	26.564	0.482	2.097
Confidence Level (95.0%)	0.048	0.027	0.076	0.133	0.037	1.646	0.412	0.585	0.037	0.167

Mean chromium concentration various from phase to phase. Regarding the maximum concentration, the highest value was detected in phase one samples (0.648 mg/L) while in phase two and three we have almost the same result (0.474 mg/L vs 0.481 mg/L). Mean magnesium concentration differ from phase one with higher value of 3.064 mg/L, to lower value in phase two 1.56 mg/L and phase three 1.221 mg/L. Zinc minimum concentration was detected in phase three (0.012 mg/L), and maximum zinc concentration was detected in phase one (0.914 mg/L) with mean concentration varying from 0.291 mg/L in phase one, to 0.251 mg/L in phase two and lowest mean concentration in phase three (0.048 mg/L). Because numerous biological and environmental samples contain low concentration of zinc, it is not difficult to contaminate them (Guyo *et al.*, 2009). Studies also demonstrated that Zn concentrations in mammary tissue were positively correlated with the milk performance and age of dairy cows (Olsson *et al.*, 2001). Potassium mean concentration various from 33.909 mg/L (phase one) and 31.242 mg/L (phase two), with lowest mean concentration in phase three 19.085 mg/L. Minimum concentration was detected in phase three (0.139 mg/L) and maximum concentration was detected in phase one 42.138 mg/L.

Table 2. Mean, minimum, and maximum contents of selected elements in milk samples – Phase II

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Mean ±SD	0.726±0.34	0.361±0.09	0.278±0.117	1.565±0.15	0.251±0.13	31.24±6.02	7.15±3.27	14.83±8.86	0.236±0.06	1.625±0.67
Median	0.78	0.166	0.33	2.135	0.262	32.64	8.093	19.358	0.247	1.534
Minimum	0.218	0.026	0.056	0.072	0.041	5.078	0.067	1.682	0.109	0.511
Maximum	2.042	5.085	0.474	3.227	0.722	38.024	10.852	25.419	0.346	2.844
Confidence Level (95.0%)	0.125	0.327	0.061	0.554	0.049	2.209	1.2	3.251	0.028	0.286

Sodium mean concentration values differ from phase one 9.758 mg/L to 7.15 mg/L in phase two and 6.45 mg/L in phase three, with minimum concentration in phase two (0.067 mg/L) and maximum concentration in phase one (12. 174 mg/L), which do not differ much from phase two and three (10.852 mg/L, respectively 11.309 mg/L). Calcium minimum and maximum concentration was detected in phase three 0.303 mg/L respectively 35.092 mg/L, with mean concentration various from 20.217 mg/L in phase one, followed by 14.838 mg/L in phase two and 17.035 mg/L in phase three. Calcium concentration in milk, similarly to the Mg content from high-producing cows often falls below the lower reference limits (Zamberlin *et al.* 2012, Litwinczuk *et al.,* 2018). Base on many authors, milk from cows which are suffering from mastitis, has lower calcium proportion than milk of the highest cytological quality (Kowalczuk *et al*., 2007, Górska *et al.*, 2012, Bilandžić *et al.*, 2011).

Table 3. Mean, minimum, and maximum contents of selected elements in milk samples – Phase III

	Fe mg/L	Cu mg/L	Cr mg/L	Mg mg/L	Zn mg/L	K mg/L	Na mg/L	Ca mg/L	Cd mg/L	Pb mg/L
Mean ± SD	0.467±0.27	0.170±0.02	0.073±0.02	1.221±0.24	0.048±0.08	19.09±9.08	6.45±1.67	17.03±7.87	0.313±0.09	2.277±1.07
Median	0.477	0.206	0.103	1.897	0.051	22.203	5.832	18.604	0.337	2.282
Minimum	0.074	0.059	0.022	0.048	0.012	0.139	4.231	0.030	0.100	0.367
Maximum	1.041	0.379	0.481	4.479	0.206	27.739	11.309	35.092	0.426	3.803
Confidence Level (95.0%)	0.105	0.077	0.089	0.549	0.031	3.455	0.635	2.996	0.034	0.41

Regarding the concentration of toxic heavy metals (Cd and Pb) in milk samples during three phases are given in Figure 2 and mean, minimum and maximum concentrations in Table 2, 4 and 6.

Mean concentration of cadmium and lead in phase one was 0.318 mg/L, respectively 1.293 mg/L, followed with mean concentration for cadmium in phase two (0.236 mg/L) and lead (1.625 mg/L). Higher mean concentration was detected for lead in phase three (2.277 mg/L), while for cadmium we have almost the same mean concentration in phase three as in two previous phases (0.313 mg/L). Minimum cadmium detected concentration was almost the same in all three phases (0.104 mg/L; 0.109 mg/L; 0.100 mg/L) while maximum concentration of cadmium various from 0.482mg/L in phase one, to 0.346 mg/L in phase two and 0.426 mg/L in phase three. Lead minimum concentration was detected in phase one with 0.144 mg/L, followed with phase two 0.511 mg/L a phase three 0.367 mg/L. Maximum concentration of lead was detected in phase three with 3.803 mg/L, while in phase one maximum detected value was 2.097 mg/L followed with 2.844 mg/L in phase two. These results are higher than values reported by Simsek *et al*. (2000).

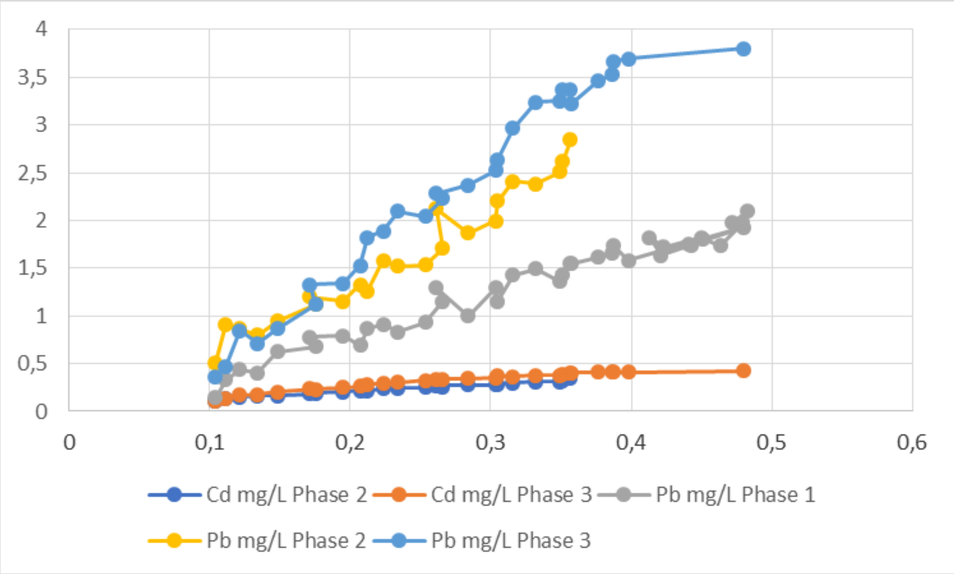

Figure 2. Concentration of Cd and Pb (mg/mL) in three phases

Table 4. Correlation between heavy metals and nutrition elements in Phase I

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Fe (mg/L)	1									
Cu (mg/L)	**0.875**	1								
Cr (mg/L)	**0.735**	**0.752**	1							
Mg (mg/L)				1						
Zn (mg/L)	0.192	0.155	0.183		1					
K (mg/L)	0.121	0.077			0.169	1				
Na (mg/L)				0.299	0.073		1			
Ca (mg/L)			0.023	**0.54**			0.117	1		
Cd (mg/L)	**0.876**	**0.929**	**0.806**		0.171	0.051			1	
Pb (mg/L)	**0.829**	**0.923**	**0.761**		0.132	0.022			**0.981**	1

The values indicate significance at $p < 0.001$

The Pearson correlation matrix, provided in Table 4, 5 and 6, shows significant correlation between heavy toxic metals and nutritional elements selected for analysis in each phase of sampling. In phase one iron is strongly positive correlated with cadmium and lead (r=0876, respectively r=0.829), and copper with cadmium and lead (r=0.929, respectively r=0.923). Also, in phase one cadmium and lead revealed high positive correlation with chromium and calcium.

Table 5. Correlation between heavy metals and nutrition elements in Phase II

	Fe (mg/L)	Cu (mg/L)	Cr (mg/L)	Mg (mg/L)	Zn (mg/L)	K (mg/L)	Na (mg/L)	Ca (mg/L)	Cd (mg/L)	Pb (mg/L)
Fe (mg/L)	1									
Cu (mg/L)	0.15	1								
Cr (mg/L)	0.291	0.289	1							
Mg (mg/L)		0.097	0.021	1						
Zn (mg/L)	0.199	0.116	0.343	0.11	1					
K (mg/L)		0.182		0.26		1				
Na (mg/L)	0.073	0.139	0.071	0.107	0.393		1			
Ca (mg/L)				0.207		0.268		1		
Cd (mg/L)	0.131	0.334	**0.976**	0.215	0.132				1	
Pb (mg/L)	0.253	0.421	**0.949**	0.237	0.204				**0.973**	1

The values indicate significance at p < 0.001

In phase two regarding heavy toxic metals and nutritional elements, the Pearson correlation matrix revealed significant positive correlations between cadmium (r=0.976), and lead (r= 949) with chromium.

Table 6. Correlation between heavy metals and nutrition elements in Phase III

	Fe mg/L	Cu mg/L	Cr mg/L	Mg mg/L	Zn mg/L	K mg/L	Na mg/L	Ca mg/L	Cd mg/L	Pb mg/L
Fe mg/L	1									
Cu mg/L	**0.689**	1								
Cr mg/L	**0.861**	**0.634**	1							
Mg mg/L		0.127		1						
Zn mg/L	0.354	0.446	0.413	0.258	1					
K mg/L				0.382	0.095	1				
Na mg/L	0.001	0.062	-0.07	0.325	0.031	0.017	1			
Ca mg/L				0.156		0.04	0.261	1		
Cd mg/L	**0.703**	**0.642**	**0.894**		**0.513**				1	
Pb mg/L	**0.788**	**0.647**	**0.949**		0.481				**0.978**	1

The values indicate significance at p < 0.001

The data in Table 6 indicate significant positive correlations (Pearson coefficients) between heavy toxic metals and nutritional elements. Cadmium and lead shows strong positive correlation with iron (r=0.703; r=0.788) and chromium (r=0.894, r=0.949). Cadmium revealed moderate positive correlation with cooper (r=0.642) and zinc (r=0.513).

DISCUSSION

The statistical analysis shows difference in the concentration of nutritional elements and cadmium and lead depending on the sampling time as shown in Figure 2. Iron concentration in phase one was 0.314 mg/L-0.836 mg/L, in phase two concentration various from 0.218 mg/L to 2.042 mg/L. and in phase three iron concentration various from 0.047 mg/L to 1.041 mg/L, which are higher maximal concentration compared with result presented by Pilarczyk *et al.* (2013).

The mean value of iron in all three phases was higher than results represent by Pilarczyk *et al*. (2013) which various from 0.1984 mg/L to 0.2576 mg/L. Copper mean concentration as nutritional elements varies from phase to phase, with maximum concentration detected in phase II (5.085 mg/L) which is higher concentration of copper in milk samples than results present by Mahlat *et al*. (2012) (2.836 µg/g). Chromium concentration various from phase to phase, but it was lower than mean results presented by Cocho *et al*. (1992).

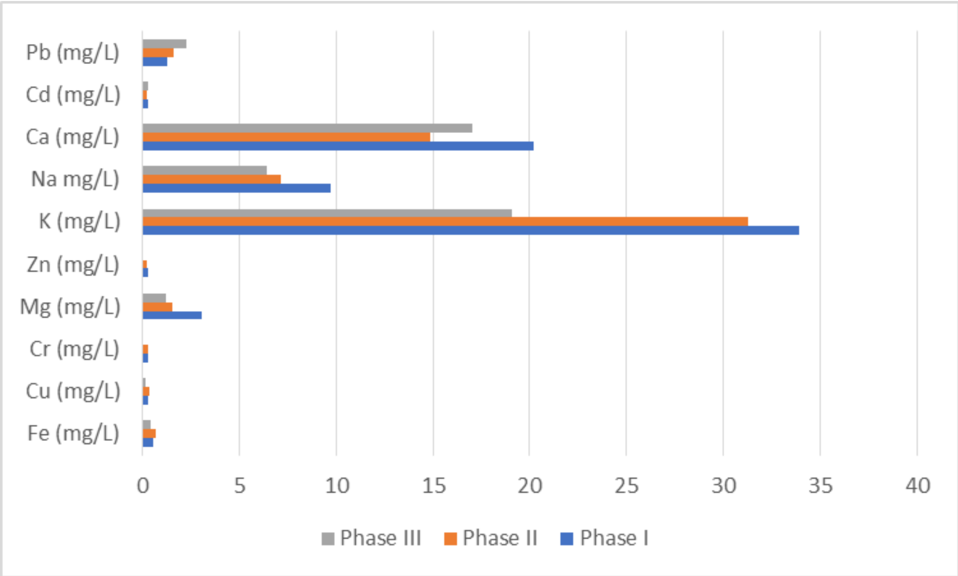

Figure 3. Mean concentration of selected elements distributed in three phases

Mean magnesium concentration differ from phase one with higher value of 3.064 mg/L, to lower value in phase to 1.56 mg/L and phase three 1.221 mg/L, which are higher than results presented by Gaucheron (2005) which are 1.043–1.283 mg/L. Zinc maximum concentration and mean values were lower than those presented by Malhat *et al.* (2012). Potassium maximum mean concentration was detected in phase one 33.909mg/L (phase one), which is lower significantly than those presented by Gaucheron (2005) 1212–1681 mg/L. Also, sodium content with higher mean concentration in phase one (9.758 mg/L) was much lower than previous results presented by Gaucheron (2005) which various 391–644 mg/L. Calcium maximum concentration was detected in phase three 35.092 mg/L which is significantly lower than results presented by Nogalska *et al.* (2020).

Mean nutritional content in the milk samples in all location sites in phase one followed the profile K > Ca > Na > Mg > Fe > Cr > Cu > Zn. In phase two nutritional elements follow almost the same profile: K > Ca > Na > Mg > Fe > Cu > Cr > Zn. In phase three nutritional elements follow this rank K > Ca > Na > Mg > Fe > Cu > Cr > Zn. Based on the above results the nutritional elements follow almost the same profile. This is also the same profile as presented by Naeem *et al.* (2022), in which zinc is switched with iron.

Regarding the toxic heavy metals, there was no significant seasonal variations in cadmium content, compared to reports by Kozhanova *et al.* (2021) where was detected significant seasonal variations in cadmium content in animal milk. In all three seasons the maximum level and mean value of cadmium and lead exceeds the values recommended in these reports and indicate a concentration in toxic values (IDF Standard, 1979, Codex Alimentarius Commission, 2007). Mean heavy metal content in the milk samples in all location sites in phase one followed the profile lead > cadmium. Correlations between milk components are presented in each phase in Table 4, 5 and 6.

A strong correlation between heavy metals and nutritional elements in cow's milk, in all three phases are observed respectively for cadmium and lead with iron, copper and chromium, moderate correlation with magnesium and zinc. The results show a very high correlation in iron and cadmium compared to cases in plants (Bajraktari *et al.,* 2020). There is noted in phase one besides a correlation for heavy metals and nutrients elements, a high negative correlation between iron with copper (r=-0.875), high positive correlation between iron and chromium (r=0.735) and copper and chromium (r=0.752), moderate positive correlation between magnesium and calcium (r=0.540), and very high positive correlation between cadmium and lead (r=0.981). In phase two, besides high positive correlation of chromium with cadmium and lead, there is detected a very high positive correlation between cadmium and lead (r=0.973), and very high negative correlation between lead and chromium (r=-0.949). In addition to heavy metal and nutritional elements correlations, in phase three there is noted a very high positive correlation between cadmium and lead (r=0.978), iron with chromium and copper (r=0.861, r=0.689), copper with chromium (r=0.634). Correlations between nutritional milk elements and toxic metals were rarely analyzed (Rodríguez *et al.,* 1999). As in the milk of Simmental and Holstein-Friesian cows, where significant high positive correlations were found between the concentrations: Pb–Cd (r=0.86 vs. r=0.87), during three phase of study also strong positive correlation between cadmium and lead was detected (Pilarczyk *et al.,* 2013).

CONCLUSIONS

The aim of this study was to analyze the content of nutritional elements in various seasonal sampling time and heavy toxic metals in cow's milk in highly polluted industrial area and to compare the results of previous studies. Results suggested that cadmium and lead concentration was higher than the recommended standards and compared with results of milk from other parts of the world. The present research showed that cow's milk in three phases of sampling had advantageous nutritional composition and higher toxic heavy metal concentration. The established correlations showed high significantly positive correlation between toxic heavy metals (cadmium and lead) in all three with chromium, in phase one and three with iron, copper. The comparative analysis in this study suggests taking the necessary measures for the production of safe food products of animal origin.

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